2.2.2. Effectiveness of Antimicrobial Preservatives.

NOTE – The test for effectiveness of antimicrobial preservatives is non-mandatory and is not intended for use for routine control purposes.

The efficacy of antimicrobial preservation of a pharmaceutical preparation on its own or, if necessary, with the addition of a suitable preservative has to be ascertained during the development of the product. The primary purpose of adding antimicrobial preservatives to dosage forms is to prevent adverse effects arising from contamination by microorganisms that may be introduced inadvertently during or subsequent to the manufacturing process. However, antimicrobial agents should not be used solely to reduce the viable microbial count as a substitute for good manufacturing procedures. There may be situations where a preservative system may have to be used to minimize proliferation of microorganisms in preparations that are not required to be sterile. It should be recognized that the presence of dead microorganisms or their metabolic by-products may cause adverse reactions in sensitized persons.

Any antimicrobial agent may show the protective properties of a preservative. However, for the protection of the consumer the concentration of the preservative shown to be effective in the final packaged product should be considerably below the concentrations of the preservative that may be toxic to human beings.

The following tests are provided to demonstrate, in multiple dose parenteral otic, nasal, ophthalmic, oral and topical products made with aqueous bases or vehicles, the effectiveness of any added preservatives, during the shelf-lives of the preparations to ensure that the antimicrobial activity has not been impaired by storage. The tests apply only to the product in the original, unopened container in which it was supplied by the manufacturer.

The test consists of challenging the preparation in its final container with a prescribed inoculum of suitable microorganisms, storing the inoculated product at a prescribed temperature, withdrawing samples from the container at specified intervals of time and counting the organisms in the samples removed. The preservative properties of the product are considered adequate if, in the conditions of the test, there is a significant fall or no increase in the number of microorganisms in the inoculated preparation after storage for the times and at the temperatures prescribed.

The organisms specified for use in the tests are intended to be representative of those that might be expected to be found in the environment in which the preparation is manufactured, stored and used. However, they should be supplemented by other strains or species, especially those likely to be found in the conditions under a particular product is made or used, or that might offer a particular challenge to the type of product being tested. Single strain challenges (rather than mixed cultures) should be used throughout.

Precautions. Challenge tests should be conducted under conditions that prevent accidental contamination of the product during the test but the precautions taken to prevent contamination should not affect the survival of organisms in the product being examined.

Test organisms. The following test organisms are used in the test.

- Candida albicans, ATCC 10231
- Aspergillus niger ATCC 16404
- Escherichia coli ATCC 8739
- Pseudomonas aeruginosa ATCC 9027
- Staphylococcus aureus ATCC 6538
In order to prevent any phenotypic changes in the strains used, the organisms used in the test should not be more than 5 passages made from the original culture. One passage is defined as inoculation and growth of the organisms from existing culture to a fresh medium.

**Media** - All the media used in the tests should be tested for growth promotion.

**Preparation of inoculum.** Grow each of the bacterial species separately in Casein soyabean digest agar and incubate them at 30 °C – 35°C for 18 to 24 hours. Grow *Candida albicans* on Sabouraud dextrose agar and incubate at 20 °C – 25°C for atleast 48 hours. Grow *Aspergillus niger* on Sabouraud dextrose agar at 20 °C – 25°C for 5-7 days. After incubation, harvest the growth and resuspend each of the organisms separately in Sterile Saline to obtain a microbial count of 1x10⁸ cfu per ml. To suspend spores of *Aspergillus niger* 0.05% polysorbate may be added to the saline. Use suspension of these organisms within 2-4 hours. The suspension may be stored at 4 °C – 8°C for a validated period of time.

**Procedure.** If sufficient volume (atleast 20 ml) of product is available in each container and the product container can be inoculated aseptically then the test can be conducted in five original containers of the product. If filled volume is less, or the container cannot be inoculated aseptically then transfer (atleast 20 ml) the product in each of five suitable sterile containers. Inoculate each container with one of the prepared and standardized inoculum in such a way that after inoculation the final concentration of the organisms remains between 1x10⁵ and 1x10⁶ cfu per ml. and the volume of the inoculum does not exceed 1% of the volume of the product. Determine the number of viable organisms by plate-count method in each inoculum suspension and from there calculate the initial concentration of microorganisms per ml of product being examined. Incubate the inoculated containers at room temperature. Determine the viable count by plate-count method at 7, 14, and 28 days subsequent to the inoculation. Record any changes observed in the appearance at these intervals. From the calculated concentration of cfu per ml present at the start of the test, calculate the percentage of reduction in cfu per ml for each organism at the stated test intervals and express the changes in terms of percentage of initial concentration.

**Interpretation** The preservatives are considered to be effective if

(i) For parenteral, opthalmic, sterile nasal and otic preparations: (a) the concentration of the viable bacteria are not more than 10% of the initial concentration at 7 days and not more than 0.1% of the initial concentration at 14 days and there is a further decrease in count at 28 days. (b) there is no increase in yeast and mold count at 7, 14 and 28 days from the initial count.

(ii) For topical preparations made with aqueous base, non-sterile nasal preparations and emulsions including those applied to mucous membrane : (a) the concentration of the viable bacteria are not more than 1% of the initial concentration at 14 days and there is a further decrease in count at 28 days. (b) there is no increase in yeast and mold count at 14 and 28 days from the initial count.

(iii) For oral preparations : (a) the concentration of the viable bacteria are not more than 10% of the initial concentration at 14 days and there is a further decrease in count at 28 days. (b) there is no increase in yeast and mold count at 14 and 28 days from the initial count.

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